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Circulatory Effects of d-lysergic Acid Diethylamide (LSD-25)

By
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There are certain drugs that can produce hallucinations and other symptoms similar to those of schizophrenia. Among these is d-lysergic acid diethylamide (LSD-25), which is a very powerful drug and the one most commonly used to produce experimental psychosis. LSD-25 is chemically related to the ergot alkaloids (HOFMANN and STOLL 1943). Its psychogenic effects were first discovered in 1943 by HOFMANN (see W. A. STOLL 1947).

The way in which hallucinations are produced is not known. The evidence argues in favour of some kind of metabolic functional disturbance in the nerve cells (cf. GLEES 1961).

The present investigation was started for the following two reasons. Firstly, I assumed, like INGVAR and SÖDERBERG (1956), that a functional disturbance of the nerve cells caused by LSD-25 could be accompanied by circulatory changes in the brain. Secondly, several recent investigators (ÅSTRÖM and SAMELIUS 1957; GINZEL 1955, 1956; see also Delysid Bibliographia Sandoz, 1961) have found that LSD-25 has various vasoactive properties.

Material

Twelve rabbits and 20 mice were used. The rabbits weighed 1280 to 1320 g. The mice weighed only 5.0—5.5 g and belonged to four litters. The rabbits were divided into one test group and one control group, each comprising 6 animals. In the same way, the mice were divided into two equal groups. The groups were made up so that the sexes and the litters were equally represented among the test animals and controls.

Methods

In order to prevent the daily variations of the liver metabolism (FORSGREN 1927; HOLMGREN 1936) from influencing the results, it was arranged that all animals should be killed between 3 and 4 p.m.

Rabbits

The animals in the test group received an intravenous injection of 5 mg of LSD-25 (Sandoz) diluted in 3 ml of a 1% aqueous solution of tartaric acid. The rabbits in the control group got an injection of 3 ml of the 1% tartaric-acid solution without LSD-25. One half of the control and test rabbits was killed 30 min after the injection, while the other half was killed 90 min after the injection. The animals were killed instantaneously in a large guillotine. The heart was then removed as quickly as possible. The heads were freed from skin, muscles, jaws, eyes, and ears, so that only the cranium and its contents remained. The olfactory bulbs were exposed by removing bone, so that the fixative could more easily reach the brain. The cranium was then placed in 15% formalin, containing 50 g of potassium ferricyanide per litre. The hearts, lungs, livers, spleens and kidneys were removed from the animals. During the dissections care was taken to loose no blood from the organs, which were gently transferred to the fixative mentioned above. After fixation, the organs were weighed and embedded in paraffin. The brains were sectioned serially in the frontal plane into 5-μ-thick sections. Every 49th and 50th section was mounted and the others were discarded. From the livers, right lungs, and left kidneys five sections, 5 μ thick, were produced from various parts of the organs. All these sections were stained with Pickworth's peroxidase stain, as modified by PEARSE (1954). Neutral red served as a counter-stain, which demonstrated the cell nuclei and allowed identification of various areas of the brains. The dark-blue-stained erythrocytes were counted under a magnification of 900×. Only capillary blood corpuscles were counted, and fields of vision with large blood vessels were avoided (GYLLENSTEN, JALLING and TIDEN 1959). A squared graticule was placed in the focal plane of the ocular (WESTMAN and HELLMAN 1960). A volume of 0.0013 mm^3, selected at random, was counted from the right lungs, livers, left kidneys and the following parts of the brains: regio praecentralis granularis, regio postcentralis, regio parietalis 4 and area striata of the cerebral cortex, capsula interna, corpus callosum, nucleus caudatus, cornu Ammonis, and the molecular, granular and medullar layers of the cerebellum. When determining the regions of the cerebral cortex, the maps made by LUNDBERG (1960) were used. Sections, 10 μ thick, were produced from the hearts and the livers and stained by van Giesons's method.

Mice

In order to freeze the mice quickly, all hairs were removed by cutting with a pair of scissors. The animals of the test group received an intraperitoneal injection of 0.03 mg of LSD-25 diluted in 0.5 ml of a 1% aqueous solution of tartaric acid. The mice in the control group got an

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injection of 0.5 ml of the 1% tartaric-acid solution without LSD-25. One hour after the injection, the animals were put into a bath isopentane cooled to about −190°C with liquid nitrogen or oxygen. Pilot experiments showed that the whole body was frozen within approximately 2 sec. In a cool room at a temperature of −30°C, the bodies were sawed into 0.5 to 1-cm-thick slices which were put into a mixture of nine parts of pure methanol (absolutely free from acetone, which causes haemolysis) and one part of 37% formalin. The slices were then kept in the mixture at −30°C for 14 days. During this time the mixture was changed once. The temperature was then raised to +4°C and the slices were transferred to 10% formalin for one day. The lungs, livers, spleens and kidneys were dissected free and weighed. Slices were cut from the heart, right lung, liver, and left kidney. Dehydration was performed on the usual ethanol scale for 24 hours. The embedding was done in soft paraffin at 40°C for 3 hours. The specimens were cast in hard paraffin (M.P. 56—58°C). One section, 5 μ thick, was produced from five different levels of the brain, lung, liver and kidney slices. The sections were stained with the peroxidase stain mentioned above. (During the course of the investigation, it was found that a better staining of the erythrocytes was obtained on sections cut on a freeze microtome or a cryostate and stained according to LINDGREN 1940.) The erythrocytes were counted as described for the rabbits, but only the regio praecentralis granularis and area striata of the cerebral cortex, the molecular, granular and medullar layers of the cerebellum, and the nucleus caudatus were assessed. Sections, 10 μ thick, were produced from the hearts and the livers and stained with van Gieson's stain.

Results

Almost immediately after the administration of LSD-25, the rabbits exhibited clear behavioural changes. They lost tonus and lay staring in front of them. The mice showed similar but less striking changes in behaviour.

As appears from Table 1, no change in the erythrocyte contents of the various parts of the brain was encountered after the administration of LSD-25. On the other hand, the erythrocyte contents of the liver were much greater in the animals treated with LSD-25 than in those not treated. When the increase in liver erythrocyte contents was tested statistically with the aid of Student's t-test (BONNIER and TEDIN 1957; SNEDECOR 1957), it was found to be highly significant ($p < 0.001$) in both the rabbits and the mice. There were also highly significant ($p < 0.001$) increases in the liver weights of the rabbits and mice (Table 2). The increase in erythrocyte contents and weight was largely the same in the rabbits killed 30 min after the LSD administration as in those

Table 1. *Erythrocyte contents of the liver, lung, kidney and various parts of the brain in animals treated and not treated with LSD-25*

	Erythrocyte contents in millions/mm³					
	Rabbits				Mice	
	LSD 30 min	Control	LSD 90 min	Control	LSD	Control
Liver	1.701 ± 0.209	0.376 ± 0.067	1.745 ± 0.298	0.341 ± 0.075	1.663 ± 0.201	0.278 ± 0.059
Lung	0.783 ± 0.101	0.796 ± 0.193	0.769 ± 0.121	0.595 ± 0.091	0.599 ± 0.114	0.542 ± 0.099
Kidney	0.610 ± 0.100	0.646 ± 0.082	0.602 ± 0.089	0.549 ± 0.079	0.493 ± 0.067	0.444 ± 0.055
R. praecent. gran.	0.140 ± 0.019	0.151 ± 0.021	0.139 ± 0.015	0.135 ± 0.020	0.130 ± 0.013	0.125 ± 0.018
R. postcent.	0.132 ± 0.022	0.140 ± 0.017	0.129 ± 0.013	0.125 ± 0.017		
R. pariet. IV	0.141 ± 0.024	0.125 ± 0.020	0.121 ± 0.018	0.133 ± 0.018		
A. striata	0.126 ± 0.016	0.139 ± 0.014	0.144 ± 0.023	0.128 ± 0.016	0.119 ± 0.015	0.099 ± 0.011
Caps. int.	0.061 ± 0.007	0.049 ± 0.005	0.054 ± 0.008	0.059 ± 0.011		
Corp. call.	0.052 ± 0.006	0.055 ± 0.008	0.058 ± 0.007	0.051 ± 0.008		
N. caudatus	0.119 ± 0.017	0.099 ± 0.011	0.108 ± 0.013	0.106 ± 0.014	0.107 ± 0.011	0.112 ± 0.010
Corn. Amm.	0.077 ± 0.011	0.072 ± 0.009	0.070 ± 0.008	0.076 ± 0.010		
Str. mol. cerebell.	0.151 ± 0.017	0.146 ± 0.015	0.139 ± 0.014	0.149 ± 0.016	0.141 ± 0.017	0.131 ± 0.015
Str. gran. cerebell.	0.190 ± 0.026	0.178 ± 0.020	0.180 ± 0.019	0.186 ± 0.021	0.167 ± 0.019	0.174 ± 0.018
Lam. med. cerebell.	0.045 ± 0.005	0.049 ± 0.007	0.043 ± 0.008	0.050 ± 0.006	0.038 ± 0.003	0.041 ± 0.005

Table 2. *Weights of the livers, spleens, lungs and kidneys of the experimental animals*

	Weight in gram after fixation					
	Rabbits				Mice	
	LSD 30 min	Control	LSD 90 min	Control	LSD	Control
Liver	121.4 ± 14.0	69.8 ± 8.1	130.9 ± 17.8	72.7 ± 9.7	0.521 ± 0.051	0.299 ± 0.031
Spleen	0.794 ± 0.173	0.506 ± 0.131	0.746 ± 0.141	0.519 ± 0.098	0.050 ± 0.012	0.038 ± 0.011
Right and left lung	19.6 ± 4.1	20.1 ± 3.3	21.9 ± 3.6	23.1 ± 4.0	0.119 ± 0.017	0.124 ± 0.019
Right and left kidney	14.1 ± 2.1	13.6 ± 1.7	13.4 ± 1.5	14.4 ± 1.9	0.134 ± 0.022	0.141 ± 0.019

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killed after 90 minutes. The sections through the livers, that had been stained according to van Gieson's method, showed no remarkable changes, except the hyperaemia. The erythrocytes could not be counted in the spleens, because they were too numerous there and could not be clearly separated from each other, even in 4-μ sections, which were the thinnest paraffin sections that could be produced with uniform thickness. The weights of the spleens were somewhat greater in the animals which had received LSD-25 than in the controls. When Student's test was applied, a slightly significant ($0.01 < p < 0.05$) difference in the spleen weight was obtained between the LSD animals and the controls. The difference was of about the same significance in the rabbit as in the mouse material. The erythrocyte contents and weights of the lungs and kidneys showed no changes. There was no dilatation of the hearts and in the sections from them no remarkable changes were observed.

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Liver	121.4 ± 14.0	69.8 ± 8.1	130.9 ± 17.8	72.7 ± 9.7	0.521 ± 0.051	0.299 ± 0.031
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Discussion

A. Comments on the Methods

There exists no method for the exact study of the blood circulation in small regional parts of the brain. Most of the classical methods for studying the cerebral circulation are either based upon direct inspection of the pia vessels or upon estimations of the total flow through the brain. However, various parts of the brain differ greatly with regard to morphology. As is well known, there are not only differences between the gray and white substances but also great variations in cytology, angioarchitecture, distribution of enzymes, etc. in the various parts of the brain. The pia vessels probably do not react like the deep intracerebral vessels to various stimuli. This is also an explanation of the divergencies in the results that often are obtained from "the pia vessel inspection methods" and "the total flow measurements" (GOTTSTEIN 1962).

Recently, INGVAR and LASSEN (1962) and SÖDERBERG (1962) have made promising attempts to investigate the regional cerebral circulation. However, like all other methods used to study the cerebral circulation, their methods have the drawbacks that they include bloody operations which may cause circulatory changes, and elicit or abolish vascular reflexes. My methods for the study of the circulation have not the disadvantages mentioned above, because no surgical intervention or anesthesia is necessary. On the other hand, my methods do not measure the rapidity of the circulation, but this may to some extent be calculated. The number of erythrocytes in the capillaries increases as a rule when their calibre increases. The calibre of the intracranial arteries and veins may be measured with high accuracy on the fixed specimen. If the pressure gradient between the large arterial and venous trunks is constant

under a given set of conditions, the volume flow through a vessel connecting them is proportional to the fourth power of its calibre (BEST and TAYLOR 1958).

My "freeze method" used for the mice is thought to be an improvement on that of SJÖSTRAND (1937). In his method, mice were put into liquid air at about −190°C and then fixed in formalin at −8° to −9°C. I used a faster freezing procedure, because I used isopentane which was not invented for this purpose when SJÖSTRAND made his investigation. I also used a lower dehydration-fixation temperature (−30°C) than that of SJÖSTRAND. These two improvements diminished the hemolysis. A rapid freezing procedure has also the advantage over a less rapid one that the risk of circulatory changes secondary to the freezing procedure is reduced. The methanol which I used has the advantage of extremely rapid penetration and water substitution, which is of importance when freeze-substitution methods are used. Methanol penetrates several times faster than ethanol or acetone (ROMEIS 1948; PERSSON 1952). Furthermore, methanol is a good fixing agent for blood (ROMEIS) and is used in several hematological methods, e.g. that of Giemsa. Unfortunately, SJÖSTRAND's and my methods are only applicable to very small animals such as the young, shaved mice, weighing only a few grams, because larger animals cannot be frozen rapidly enough to prevent haemolysis, which is easily discovered by a deformation of the individual erythrocytes and a dark discoloration of the tissues surrounding the vessels.

B. Comments on the Results

The results show that LSD-25 causes a hyperaemia of the liver. There may be various causes for this hyperaemia. It may be caused by a congestion of blood in the liver due to a backward failure of the heart. The slightly significant increase in the weights of the spleens in the LSD animals may support such an assumption. On the other hand, there were no macro- or microscopical changes in the hearts which would have caused such a backward failure, and there were no changes in the weights and erythrocyte contents of the lungs and kidneys in the LSD animals. Therefore I find it more probable that the hyperaemia of the liver was caused by some toxic influence from the LSD on the liver circulation or that the hyperaemia was a sign of a detoxifying function of a detoxifying function of the liver. BOYD (1959) and SLAYTOR et al. (1959) have provided evidence that the liver has a detoxifying function and they found that 60—80% of the LSD administered is excreted in the bile within 3 hours. In my investigation, no histological changes (except the hyperaemia) suggesting toxic damage to the liver cells were discovered with van Gieson's method. The slightly significant increase of the weight of the spleen after LSD treatment may be second-

ary to a circulatory disturbance of the liver or may be primarily due to some detoxifying function of the reticuloendothelial system of the spleen, as of that of the liver.

My results from the brain are in accordance with those of INGVAR and SÖDERBERG (1956), who only could provoke a slight, transient vasoconstriction of the cerebral vessels when LSD was injected intravenously. My investigation, which may be regarded as a complement to their physiological experiments, shows that not only the total blood flow through the brain but apparently also the regional circulation is unaffected by LSD administration.

Zusammenfassung

LSD-25 wurde Kaninchen intravenös und Mäusen intraperitoneal gespritzt. Folgende Befunde wurden erhoben: 1. Starke Hyperämie der Leber mit deutlicher Gewichtszunahme, jedoch ohne Veränderungen des Parenchyms. 2. Geringe Erhöhung des Milzgewichtes. 3. Weder makroskopische noch mikroskopische Veränderungen am Herzen, die auf einen Herzschaden hindeuten. 4. Keine Veränderungen des Erythrocytengehaltes oder des Gewichtes der Lungen und Nieren. 5. Keine Veränderungen der Erythrocytenzahl im Gewebe verschiedener Teile des Gehirnes.

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* See also Sandoz' catalogue of the literature on Delysid that is available from Clinical Research Department, Sandoz Ltd. Basle, Switzerland.

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Erratum

zu der Arbeit RAHMANN, H., und W. ENGELS, „Verhaltensphysiologische Wirkungen von Chlorpromazin bei Karauschen (Carassius carassius L.)" Psychopharmacologia (Berl.) **6**, 71—78 (1964).

In Abb. 1, f muß in der Kurve der Herbsttiere der letzte Wert (10 h) statt bei 1 mg/l eine Ordinateneinheit höher bei 2 mg/l liegen, wie dies im Text (S. 75) auch richtig erwähnt wird.

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